CLAIMS

1. A nucleotide construct comprising a sequence encoding a positive selection marker flanked by restriction enzyme sites, the restriction enzyme sites flanked by sequences which are not complementary to each other and which do not include at least one type of base at any position, wherein the construct can be treated so that single-stranded regions are created at each sequence lacking at least one nucleotide.

2. The construct according to claim 1 wherein the single-stranded regions are created by treating the vector with restriction enzymes and with a DNA polymerase.

3. The construct according to claim 2 wherein the DNA polymerase is T4 DNA polymerase.

4. A construct according to claim 1 wherein the positive selection marker is a neomycin resistance gene (Neo^r).

5. A construct according to claim 1 further comprising a screening marker on the side of the restriction enzyme sites opposite the positive selection marker.

6. A construct according to claim 5 wherein the screening marker is green fluorescent protein (GFP).

7. A construct according to claim 5 wherein the screening marker is a modified fluorescent protein.

8. A construct according to claim 1 further comprising a negative selection marker.

9. A construct according to claim 8 wherein the negative selection marker is thymidine kinase (tk).

10. A construct having the sequence of SEQ ID NO:1.

11. A construct having the sequence of SEQ ID NO:2.

12. A method of making a DNA construct useful in introducing a nucleotide sequence into a target DNA, comprising:

(a) amplifying in one reaction, a first polynucleotide comprising two different nucleotide sequences substantially homologous to the target DNA; and

(b) inserting a second polynucleotide between the two different nucleotide sequences.

13. The method according to claim 12 wherein the first polynucleotide is amplified directly from a plasmid library.

14. The method according to claim 12 wherein the second polynucleotide encodes a positive selection marker.

15. The method according to claim 14 wherein the positive selection marker is a neomycin resistance gene (Neo^r).

16. The method according to claim 12 wherein the first polynucleotide further comprises a gene encoding a selectable marker.

17. The method according to claim 16 wherein the first polynucleotide includes a sequence encoding ampicillin resistance.

18. The method according to claim 12, wherein step (a) comprises amplifying a genomic clone with oligonucleotide primers.

19. The method according to claim 18 wherein the oligonucleotide primers have 5' sequences lacking one type of base.

20. The method according to claim 19 wherein the 5' sequences lacking one nucleotide are at least 5 nucleotides in length.

21. The method according to claim 19 wherein the 5' sequences lacking one nucleotide are at least 12 nucleotides in length.

22. A method of making a DNA construct useful in introducing a nucleotide sequence into a target DNA, comprising:

(a) providing a polynucleotide substantially homologous to the target DNA;

(b) generating two different fragments of the polynucleotide;

(c) providing a vector having a gene encoding for a positive selection marker; and

(d) using ligation independent cloning to insert the two different fragments into the vector to form the construct,

wherein the positive selection marker is between the two different sequence fragments in the construct.

23. The method according to claim 22 wherein the positive selection marker is a neomycin resistance gene (Neo^r).

24. The method according to claim 22 wherein the vector is pDG2 having the sequence of SEQ ID NO:1.

25. The method according to claim 22 wherein the vector is pDG4 having the sequence of SEQ ID NO:2.

26. The method according to claim 22 wherein the vector further comprises a second sequence coding for a screening marker.

27. The method according to claim 26 wherein the screening marker is green fluorescent protein (GFP).

28. The method according to claim 26 wherein the screening marker is a modified fluorescent protein.

29. The method according to claim 22 wherein the vector further comprises a second sequence coding for a negative selection marker.

30. The method according to claim 29 wherein the negative selection marker is thymidine kinase (tk).

31. The method according to claim 22 wherein step (b) comprises PCR amplifying the fragments with oligonucleotide primers having 5' sequences lacking one type of base.

32. The method according to claim 31 wherein the 5' sequences lacking one type of base are at least 5 nucleotides in length.

33. The method according to claim 31 wherein the 5' sequences lacking one type of base are at least 12 nucleotides in length.

34. The method according to claim 31 wherein the oligonucleotide sequences are selected from the list of sequences consisting of SEQ ID NOs 3 to 10.

35. The method according to claim 22 wherein the ligation independent cloning is performed in one step.

36. The method according to claim 22 wherein the ligation independent cloning is performed in more than one step.

37. A nucleotide construct comprising a sequence encoding a positive selection marker flanked on each side by at least one restriction enzyme site, the restriction enzyme site flanked by a pair of annealing sites which do not include at least one type of base at any position, wherein none of the annealing sites have sequences complementary to other, wherein the construct can be treated to create single-stranded regions between the pair of annealing sites.

38. The nucleotide construct of claim 37, wherein the restriction enzyme site is unique.

39. A method of disrupting the function of a target sequence or gene in a cell, the method comprising:

(a) inserting sequences homologous to the target gene into the construct of claim 37 such that the sequences homologous to the target gene flank the positive selection marker, to produce a targeting construct; and

(b) introducing the targeting construct into the cell to produce a homologous recombinant wherein the function of the target gene or sequence is disrupted.

40. The method of claim 39, wherein the cell is an ES cell.

41. The method of claim 40, wherein the construct of claim 37 has the sequence of SEQ ID NO: 1 or SEQ ID NO: 2

42. The method of claim 39, wherein the homologous sequences are sequences flanking the site in the target gene that is to be disrupted.

43. The method of claim 39, wherein the targeting construct further comprises a green fluorescent protein (GFP) screening marker.

44. The method of claim 43, further comprising enriching for the homologous recombinant having the target gene or sequence disrupted, wherein the enrichment step comprises screening cells containing the targeting construct, under ultraviolet light and identifying cells that do not fluoresce.

45. A targeting construct produced by the method of claim 39.

46. The targeting construct of claim 43, wherein the construct further comprises a screening marker.

47. The targeting construct of claim 44, wherein the screening marker is green fluorescent protein (GFP).

48. A host cell containing the construct of claim 37.

49. A host cell containing the targeting construct of claim 45.

50. An animal or plant containing the construct of claim 45.